REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

I. Claim Status

Claims 1, 25 and 67 are currently being amended, and claims 72-83 have been added. Support for this amendment can be found, *inter alia*, on page 12, line 27 of the published PCT application and in Example 4 and Tables 2 and 5. Withdrawn claims 8-11 and 47-66 have been cancelled without prejudice or disclaimer thereto.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

After amending the claims as set forth above, claims 1-7, 12-32, 37-46 and 67-83 are now pending in this application.

II. Claim Rejections under 35 U.S.C. § 103

The Supreme Court recently reaffirmed the *Graham* factors for determining obviousness in *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727,1739 (2007). Further, the Court concluded that when considering the obviousness of a combination of known elements, the proper inquiry is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Id.* at 1740. On this basis, Applicant respectfully traverses the rejections discussed below.

(i) Sanford, Balhorn, Oard

Claims 1-5, 7, 12-13, 17-20, 22-30, 32, 37, 38, 42-45 were rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Sanford (U.S. Pat. No. 5,204,253) and Balhorn (*Mol. Reprod. Dev.* (2000) 56:230-234) as evidenced by Oard (*Plant Cell Tissue Organ Culture* (1993) 33:247-250). Applicant respectfully traverses this rejection for the reasons provided previously and those discussed herein.

As an initial matter, as the Examiner acknowledges, Sanford fails to teach (or even suggest) the use of arginine of the formula $[Arg]_{2-10}$ or a physiological acceptable salt thereof with regard to DNA coated metal particles. In addition, Applicant points out that Sanford also fails to address the stability of its DNA coated particles, or otherwise suggest any means for stabilizing such particles.

Of all the references cited by the Examiner, Balhorn is the only one that discusses short arginine homopolymers in any capacity. Balhorn discusses these peptides as they relate to the stability of a DNA-protamine complex (not a metal carrier particle coated with a nucleic acid), where stability (or lack thereof) relates to decondensation or dissociation of DNA from an arginine-rich peptide over a matter of seconds. *See* Balhorn, Figure 3 and Table 1. Even with regard to the disclosed toroids, however, Balhorn presents no discussion or suggestion regarding long term stability of DNA-protamine (or DNA-arginine rich peptides), as relating to a half life in days, as taught in the present application. Thus, those skilled in the art would not have thought to substitute arginine-rich peptides (such less a homopolymer of $(Arg)_{2-10}$ in particular) for spermidine and $CaCl_2$ when making the particles disclosed in Sanford, as suggested by the Examiner.

In an effort to expedite prosecution, Applicant has amended the claims to explicitly recite a surprising new characteristic of the inventive particles, namely that these particles exhibit a greater than expected stability. As shown in Table 5 of the specification, particles containing spermidine and $CaCl_2$ as a traditional condensing agent have a half life of only 3.1 days at 40° C. Further, particles formulated with $(Arg)_4$ but no chelator (formula TA201.2) also have a half life of only 3.1 days at 40° C.

Surprisingly, however, particles comprising both $(Arg)_4$ and a chelator (as in formula TA201.5, for example) have a half life of greater than 230 days at 40° C. *See* Table 5, page 13 of published application. This increased stability is also observed at 4°, 25° and 60° C (*see* Tables 2 and 5). Furthermore, shorter polymers of arginine, namely those of 4 and 6 monomers, were found to be surprisingly more stable than a polymer of about 80 monomers. (*See* Example 4, beginning on page 32 of the published application). Without being limited to this rationale, this improved stability appears to be due to improved attachment of the DNA

to the particles, as explained in Applicant's previous Reply. Therefore, short homopolymers of arginine are not merely interchangeable with spermidine as a stabilizer; they are a surprising improvement on spermidine. Adding a chelator further improves the stability of the particles as shown by its more than additive effect on their half life, which was also unexpected.

This increase in stability using shorter polymers of arginine, as required by the claimed invention, was very surprising, especially in view of what was known in the art at the time of filing the present application, such as evidenced by Adami et al., *J. Pharm. Sci.* 87:678-683 (1998) (provided herewith as Exhibit A). Adami, for example, taught that a polymer of 18 lysine monomers protected DNA from both enzymatic and sonicative-induced degradation, while a shorter polymer of 8 lysine failed to protect DNA from degradation when used as a DNA condensation agent. *See, e.g.*, Abstract and first full paragraph of page 682, left column. Like arginine, lysine is also a basic amino acid with similar chemical structure and properties. Thus, one of skill in the art would have expected that longer polymers would be required for enhanced stability upon reading Adami.

The art cited by the Examiner does not provide any basis for predicting the enhanced long term stability of the present inventive particles. Sanford teaches the use of particles onto which DNA is precipitated in the presence of EDTA, spermidine and calcium choride. The preparation of the particles and their administration takes place on the same day (*see* col. 14, lines 45-46). Likewise, Oard prepared and used its "microcarriers" "as soon as possible after precipitation because the amount of clumping increased over time." Oard, page 249, right column, first paragraph. Balhorn, cited by the Examiner as allegedly teaching the desirability of using polyarginine for improved DNA transfection, also notes the use of protamines for increasing resistance to degradation, but does not mention the effect a small homopolymer of arginine or a chelator on stability as measured in days. As discussed above, at most, Balhorn suggests that arginine peptides provide more favorable kinetics with regard to dissociation of arginine-rich peptides from DNA, as measured over a matter of seconds.

Moreover, Balhorn merely looked at the "off-rate" of the arginine peptides from the condensed DNA, concluding that the faster dissociation of arginine peptide from the DNA

may increase gene integration of the DNA after cell entry. A faster dissociation from the DNA does not suggest greater stability of the composition; indeed, it may suggest the opposite. None of the references cited by the Examiner teach nor suggest the surprising effect that using a chelator and a short homopolymers of arginine increases the stability of a nucleic acid coated particle so dramatically as disclosed in the present specification.

In sum, the claimed combination of elements has surprising results, namely the unexpected stability of the particles when a short arginine homopolymer and a chelator are present during deposition of the nucleic acid. Thus, the particles claimed herein and methods for making these particles are "more than the predictable use of prior art elements according to their established functions" per the Supreme Court's test in *KSR Int'l* as discussed *supra*, and therefore nonobvious. Applicant respectfully requests that the rejection be withdrawn.

(ii) Sanford, Balhorn, Oard, Cherng

Claims 1-5, 7, 12-15, 17-30, 32, 37-40, 42-46 were rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Sanford (U.S. Pat. No. 5,204,253) and Balhorn (*Mol. Reprod. Dev.* (2000) 56:230-234) as evidenced by Oard (*Plant Cell Tissue Organ Culture* (1993) 33:247-250) and Cherng (*Pharma. Res.* (1999) 16:1417-1423). Applicant respectfully traverses this rejection for the reasons provided previously and those discussed herein.

The Examiner applies Sanford, Balhorn and Oard as discussed previously, and cites Cherng as allegedly teaching that sucrose can stabilize condensation of nucleic acids with a cationic polymer. Applicant respectfully asserts that Cherng, alone or in combination with the other references, does not teach or suggest the surprising stability of the present particles formed in the presence of short homopolymers of arginine and a chelator.

As discussed in detail above, Sanford, Oard and Balhorn do not teach or suggest the surprising stability of the claimed particles, which are obtained by depositing a nucleic acid onto an inert metal carrier particle in the presence of $(Arg)_{2\text{-}10}$ in the presence of a metal ion chelating agent. As disclosed in the specification, particularly in the examples, these particles have a greatly increased half life as compared to traditional spermidine/calcium chloride condensed particles, and omission of either the arginine or the chelator reduces that stability.

Further, it was found that short homopolymers of arginine conferred more stability than longer homopolymers. None of the cited references teach or suggest that such a combination would confer such significant, non-additive stability, and as such the particles are nonobvious.

Cherng was cited to allegedly teach the desirability of using a sugar, namely sucrose, in the deposition so as to increase stability. Addition of a disaccharide and/or trisaccharide is an optional step to which dependent claims 14-16 and 39-41 are drawn. First, Cherng did not use metal particles in its study, instead using a polymer-plasmid polyplex. The polymer used was a methacrylate-based polymer, which is chemically very dissimilar to short homopolymers of arginine. Cherng carefully notes that the results are only applicable to the polyplexes described therein, and at best extended to polyplexes and lipid formulations (page 1423, second paragraph). Thus, one of skill in the art is explicitly cautioned from applying the conclusions made therein to other systems.

More importantly, Cherng does not provide any disclosure that would overcome the previously discussed failure of cited combination of references to teach or suggest the surprising stability of the present inventive particles. The effect of short homopolymers of arginine and a chelator on particle stability is not discussed in this reference or the others. As this stability is surprising in view of the art known at the time of filing, as discussed above, the present invention is nonobvious. Therefore, Applicant respectfully requests that the rejection be withdrawn.

(iii) Sanford, Balhorn, Oard, Cherng, Barman, Livesey

Claims 1-7, 12-32, 37-46 and 67-71 were rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Sanford (U.S. Pat. No. 5,204,253) and Balhorn (*Mol. Reprod. Dev.* (2000) 56:230-234) as evidenced by Oard (*Plant Cell Tissue Organ Culture* (1993) 33:247-250) and Cherng (*Pharma. Res.* (1999) 16:1417-1423), and in further view of Barman (U.S. Pat. Appl. Publ. No. 2004/0142475) as evidenced by Livesey (U.S. Pat. No. 6,194,136. Applicant respectfully traverses this rejection for the reasons provided previously and those discussed herein.

Sanford, Balhorn, Oard and Cherng are applied as discussed previously. Barman is cited by the Examiner as allegedly teaching that saccharides may be used to stabilize nucleic acid protein complexes, and that HPV, HIV, HBV and HSV antigens may be encoded as transgenes for the expression of antigens. Similarly, Livesey is cited as allegedly demonstrating that sugars, including raffinose, can be used as stabilizers. However, neither of these references overcome the defect of the other references, which is the failure to teach or suggest the surprising stability of the claimed particles. Barman teaches the use of polymeric microparticles, not inert metal particles, and mentions that stabilizers may be used, with examples including sugars and cationic peptides. *See* paragraphs 41 and 46. However, the use of short homopolymers of arginine and a chelator is not disclosed, nor their effect on stability of the particles. Likewise, Livesey provides only general disclosure of cryopreservatives, and does not mention inert metal particles, short homopolymers of arginine or chelators. Thus, the addition of these two references to the other references still fails to disclose surprising characteristics of the present invention. Applicant respectfully requests that the rejection be withdrawn.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing or a credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the

Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date Nov. 3, 2008 By _____

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